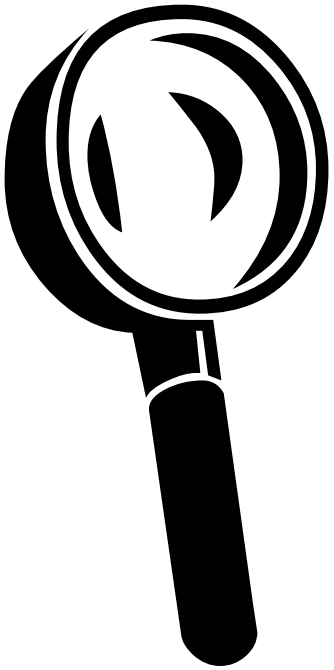


Institute for Microbiology shows

TRACING THE PATHOGEN

Part twelve:

Cooperation at investigation or Clinical Microbiology III

List of topics

Infections of urinary tract – survey

Urine sampling

Possibilities for urine specimen processing

Result interpretation, prevention and treatment of UTI

Infections of genital tract – introduction

Sampling and examination of genital infections

Urinary tract infections – survey

Importance of urinary tract infections (UTI)

- Besides respiratory infections, this is another very important group of infections with **economic losses and inconvenience for patients**
- Dangerous is **risk of complications** — for example, from cystitis and pyelonephritis may become the bearing may become the emergence of urosepsis, i. e. infection of the bloodstream
- IMC are **very common, especially in women**
- Causative agents are usually bacterial, **antibiotic therapy** is therefore often (though not always) indicated

Urinary tract of a healthy person

Kidney – without microbes normally

Renal pelvis – without microbes normally

Ureters – without microbes normally

Bladder of young and middle aged people – without microbes normally

Bladder of seniors – even under normal circumstances it can be settled in microbes, which does not make problems and becomes a "normal flora"

Urethra – normally without the microbes with the exception of the final part (bacteria from the skin + partly specific flora: viridans Streptococcus, aerococci, etc.)

Anatomic classification of UTI – 1

- **Urethritis** cases are rather **connected with genital infections** and are discussed in the context of this issue
- **Cystitis** are **the most common UTIs**, especially for women (they have a shorter urethra). They are often associated with situations where a stream of urine as a natural protection system is weaker, for example:
  - the pelvic floor disorders (typically in women after childbirth)
  - for hyperplasia of prostate (that is, by contrast, for women rare;-))
- Complications of cystitis may be **pyelonephritis**

Clinical description of cystitis

- **Burning** during urination
- **Frequent** urination, **small amounts of urine**
- Sometimes **urine is blood and turbid**
- ***In case of back pain it is usually not cystitis, but pyelonephritis***

http://www.salon.com/health/feature/1999/04/26/interstitial_cystitis/index.html

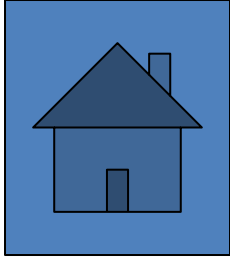
Attention – cystitis symptoms are not specific

Diseased urination (frequent urination, incontinence, burning) may have **another cause than cystitis**, which should be revealed or excluded

- It can be a **sexually transmitted disease** (*Chlamydia* or *Mycoplasma* infection, gonorrhoea)
- It can be a **non-infectious inflammation** (mechanical irritation of catheterization, etc.) or other non-infectious cause (even incipient tumour!)
- It could also be an **inflammation of the walls of the bladder** (e.g., infectious, parasitic*/Schistosoma,* as well as non-infectious)

In all these cases is finding in the urine culture is negative

Pyelonephritis



- Pyelonephritis is an **inflammation of the renal pelvis**, unlike glomerulonephritis.
  - *Glomerulonephritis affects the glomeruli and is usually non-infectious; however, it may be an autoimmune origin after a streptococcal infection.*
- It is more serious than cystitis, it **does not only affect the lumen of the urinary tract, but kidney tissue, so antibiotic therapy should respect it**
- Usually arises as a **complication of cystitis, but the origin can also be haematogenous**
- Recurrent pyelonefritis may also be complicated by **urolithiasis** (bladder stones)

Urine sampling

UTI diagnostics

- **Anamnesis**, that might also include sexual life (gonorrhoea and other urethritis)
- **Clinical** examination
- Orientation **diagnostic strip examination** (the presence of bacteria in urine)
- **Biochemical** examination – the presence of bacteria, proteins, etc.
- **Microbiological testing** is **recommended** for uncomplicated and **necessary** for complicated one (not speaking about pyelonephritis)

Sampling and transport of urine

- The most reliable urine is obtained by **suprapubic puncture.** In practice, however, rarely used
- Quite good is also **catheterized urine** (catheterization performed due to sampling)
- **Commonly collected urine sample** may not be bad, if it is properly sampled and transported
- **Urine from permanent catheter** is the worst of possible samples, sometimes, of course, there is nothing for us. Sometimes also **urine from nephrostomy** is used.

There is no reason to use cathethrized urine globally. It only makes sense when the outcome is repeatedly doubtful.

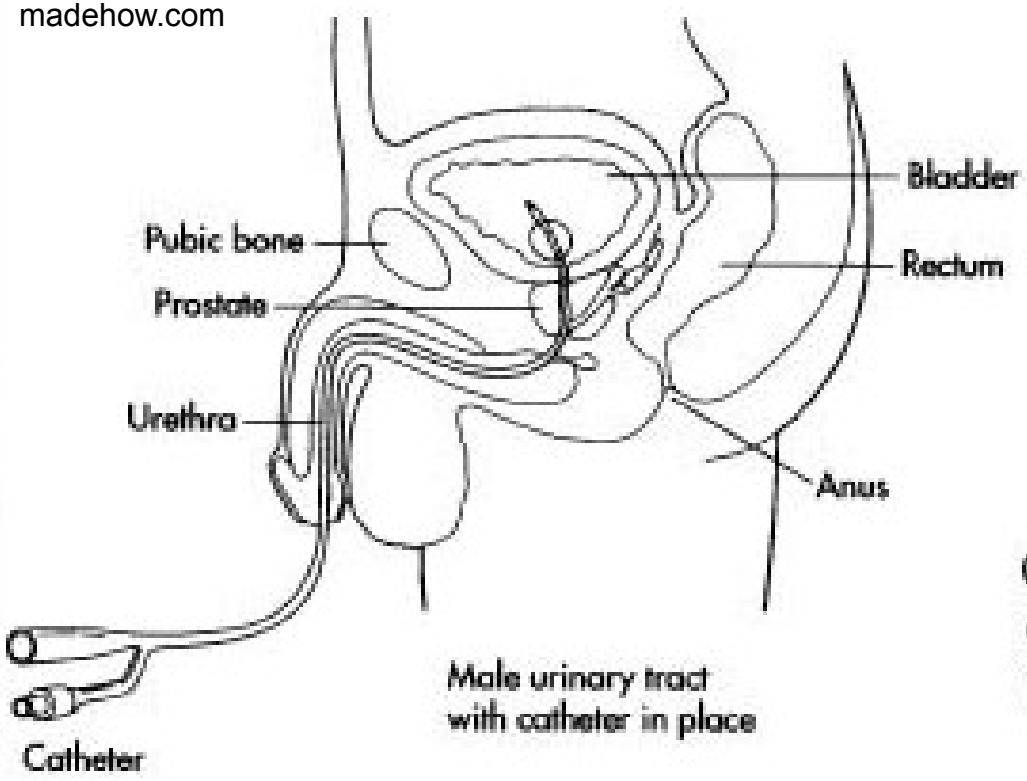
Permanent catheter urine sample

- If it is possible to **wait a few days** e. g. for the exchange of the catheter, it is better – the result of the newly exchanged a catheter will be far better (but it is advisable to wait after replacing some time until the colonizing bacteria from the old catheter are washed away)
- If we cannot wait, **we sample the urine, but we must reckon with the fact that the interpretation is ambiguous** (inflammation and not just the colonization is likely in case of leucocytes present in the urine)
- We must think whether **we are considering treatment with antibiotics at all**; If not (asymptomatic bacteriuria) the examination looks to be useless
- *Microbiological examination of the catheter itself, sent to a laboratory, is not recommended (nearly impossible interpretation), although some laboratories perform it*

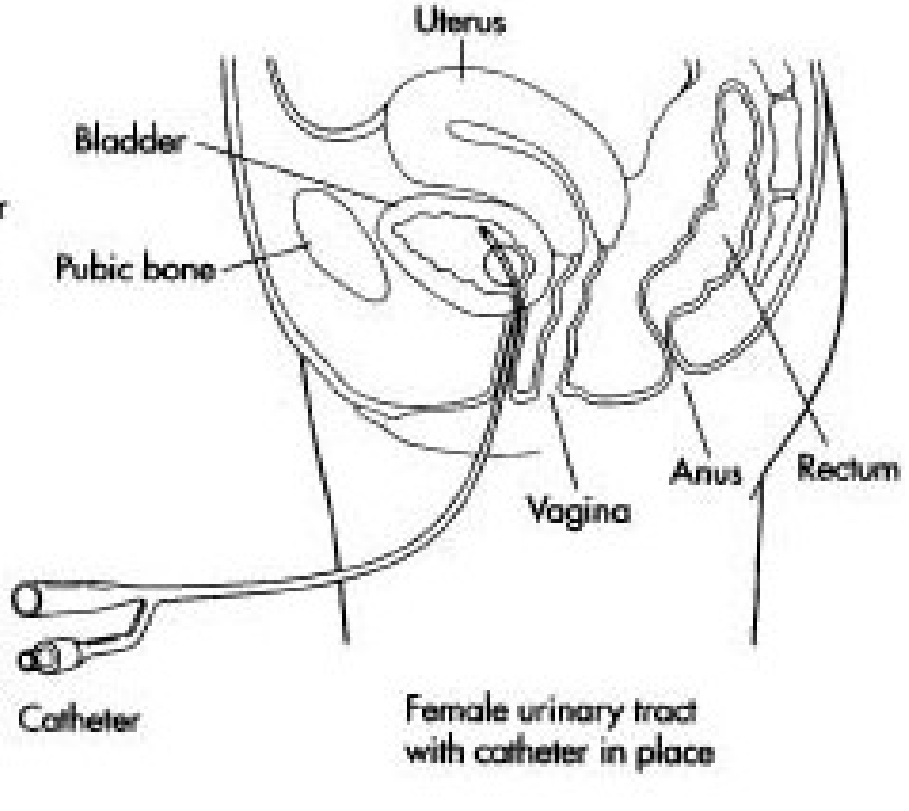
Permanent catheters

mediform.cz

madehow.com



Male urinary tract with catheter in place



Female urinary tract with catheter in place

Sampling of spontaneously urinated urine

- It is the sampling from the middle stream of urine spontaneously urinated *(routine type with secondary risk of contamination during sampling)*
- **Technique:** The container for the collection of urine must be sterile, *wide-neck (e.g. a beaker*)*, knowledgeable patient thoroughly washed prior to the collection's external genitals with soap and water and *(possibly)* wipe off the outer estuary of the urethra's swab moistened with disinfectant in *solution (especially in children, however, the use of disinfectant is not recommended)*

**this is as it is written in the official recommendations, in practice, it depends on the situation; If the patient urinates directly into a test tube, it is better*

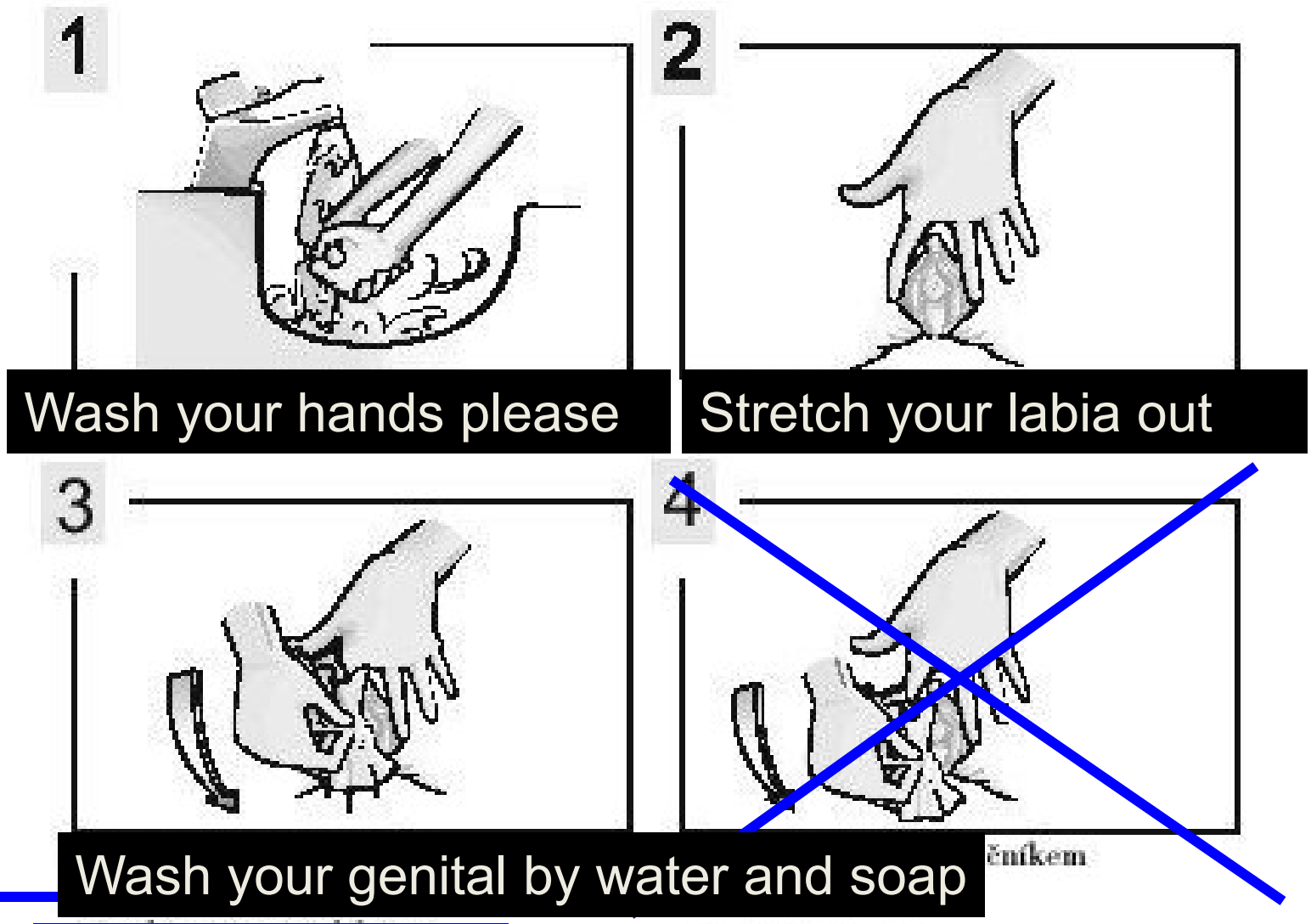
Sampling in males and females

- **Males** by one circulating movement
- **Females** should be in wide position over the toilet, they stretch out the labia by one hand and they wash the genital by a tampon using the other hand from front to back.
- After that, the patient urinates the first part of urine and **the midstream is taken into sterile vessel without breaking the urination**. *Sampled urine is placed into a sterile container for transport.*

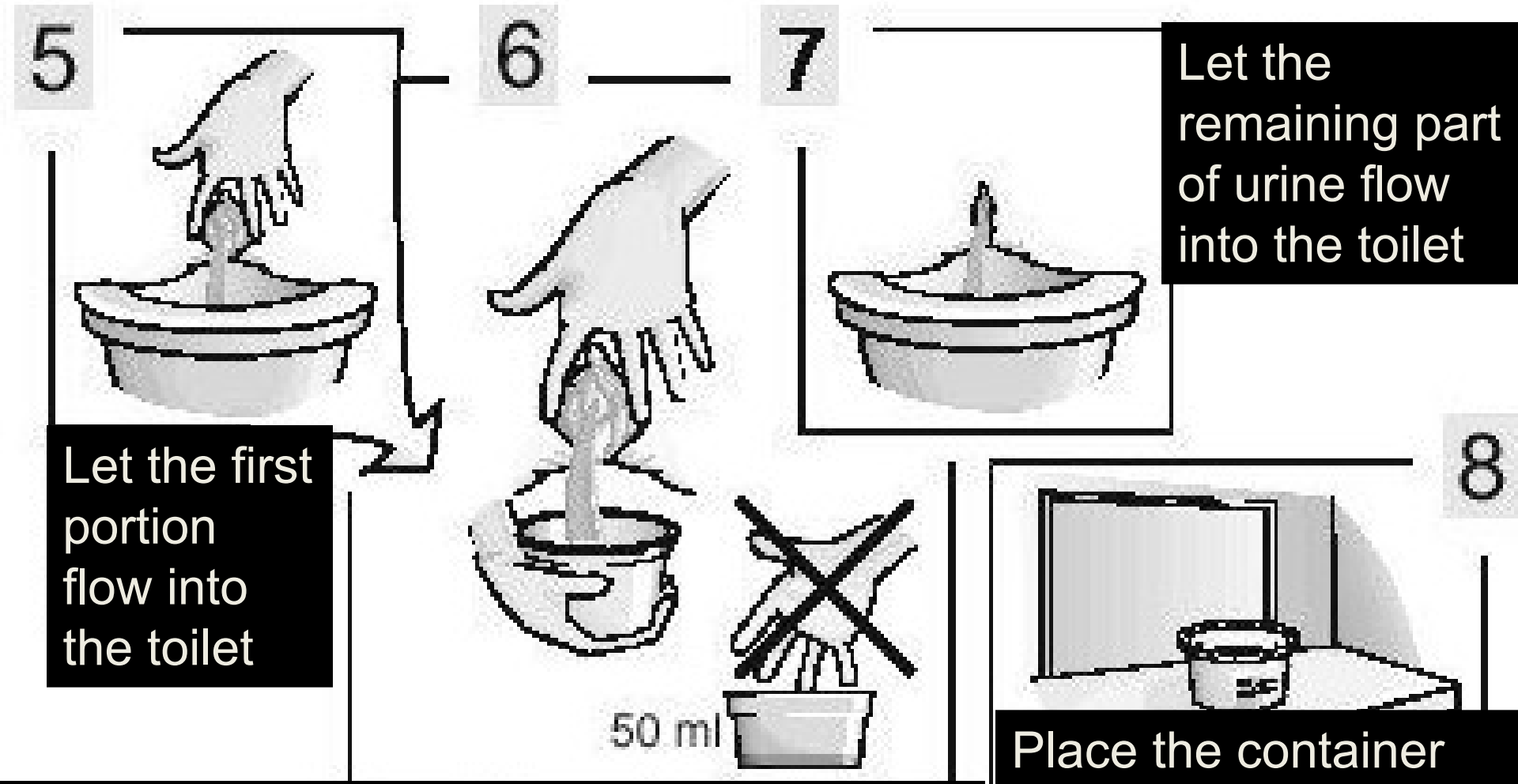
*(**Czech Medical Society of John Evangelist Purkyně, RECOMMENDED GUIDLINES FOR GENERAL PRACTICIONERS**, Project of Healthcare Ministry of Czechia by help of a grant IGA MZ ČR 5390-3)*

Urine sampling in a female – technique

http://www.lab-turnov.ic.cz/schema_1.php



Urine sampling in a female – technique



Let the first portion flow into the toilet

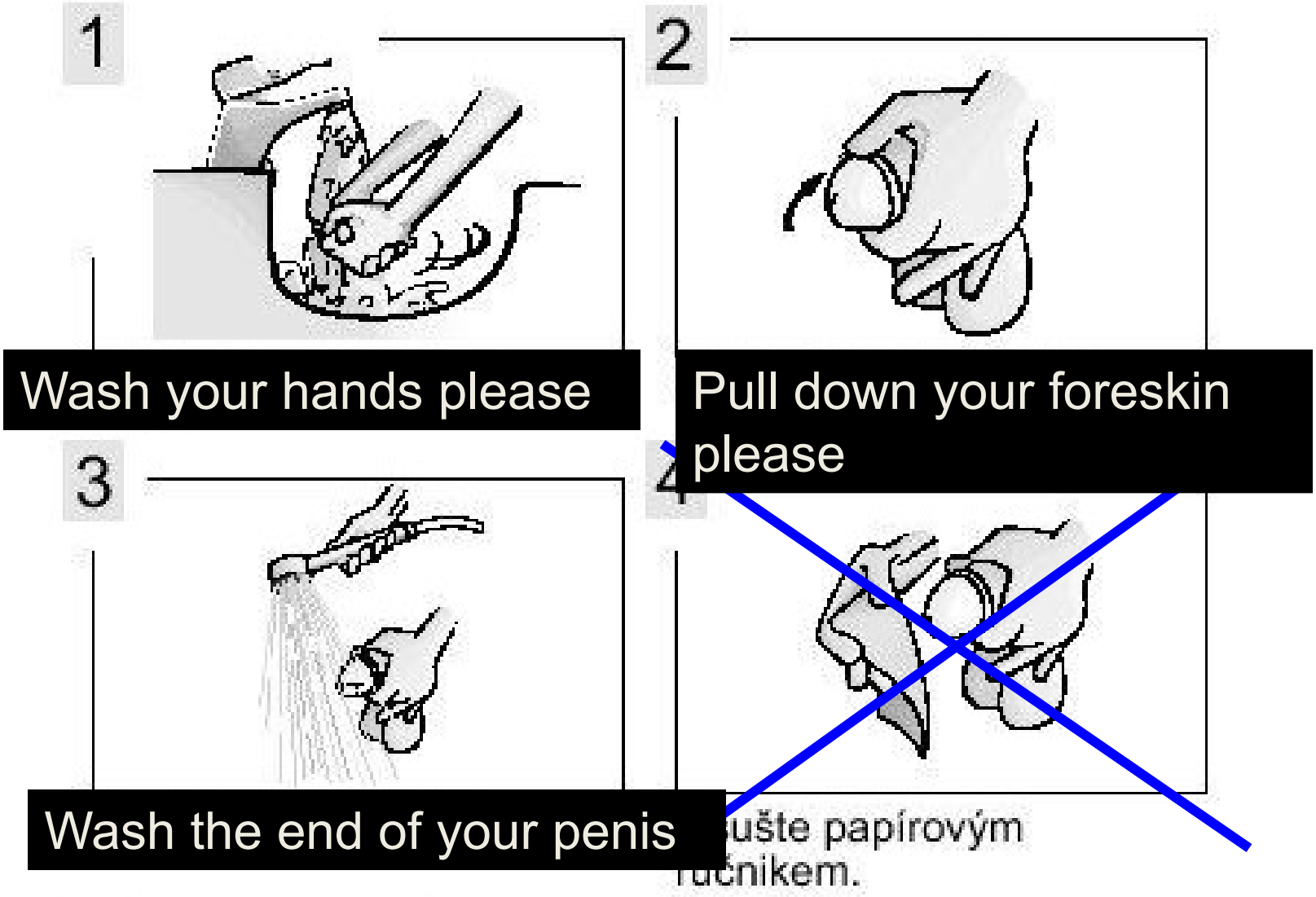
Let the remaining part of urine flow into the toilet

Catch approx. 50 ml of urine to the vessel without interrupting urination. Do not touch the inner part of the vessel.

Place the container with the urine according to directions

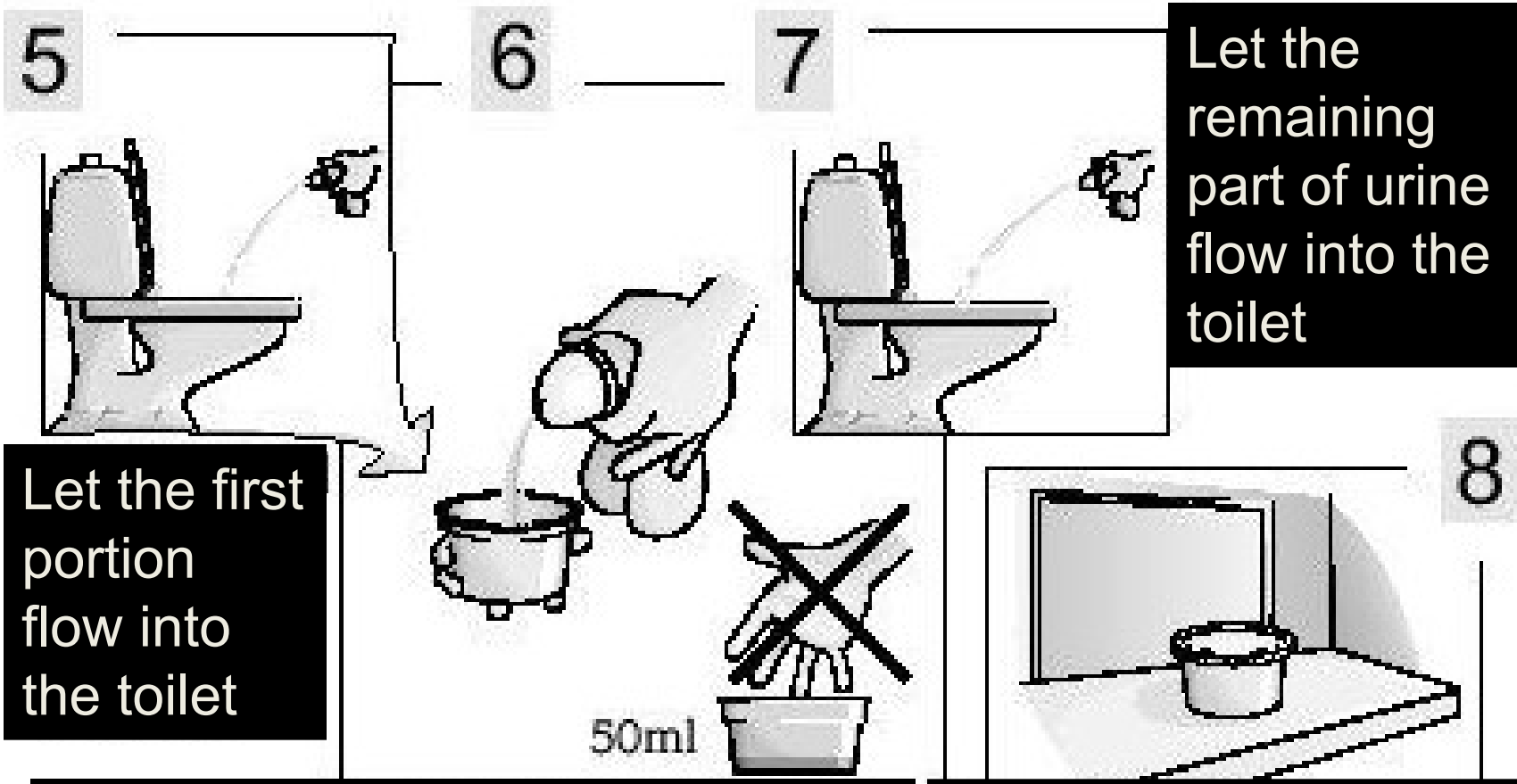
Urine sampling in a male – technique

http://www.lab-turnov.ic.cz/schema_2.php



Urine sampling in a female – technique

http://www.lab-turnov.ic.cz/schema_2.php



Let the first portion flow into the toilet

Catch approx. 50 ml of urine to the vessel without interrupting urination. Do not touch the inner part of the vessel.

Let the remaining part of urine flow into the toilet

Place the container with the urine according to directions

Exceptions of urine sampling rules

- In suspicion for urethritis we take **first portion of urine** (we wash out the microbes from urethra).
- In prostatitis we rather use **last portion of urine**
- **For schistosomosis** we collect last portions of urine several time, at least 20 ml needed. (In the laboratory, the urine will let settle and then we look for the eggs of the parasite in the sediment on the bottom). **Transport should be quick.**

Sampling in small children and catheterized patients

- **In children**
  - Urine is obtained by **collecting the bags tightly**
  - Method is burdened with a **relatively high risk of secondary contamination**
  - The bag should **not be attached more than 30 minutes**
  - It should be removed **immediately after the pee**
- **In catheterized patients** we should reckon with the fact that any result is indicative for the colonization of the catheter, rather than for the infection. The sampling must be carried out so as to minimize the risk of further contamination

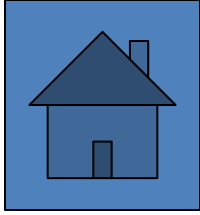
Urine transport

- For evaluation of an UTI, the **quantity** is important – see further. The quantity can only be evaluate if the microbes would not multiply in the urine during the transport. If they do, the quantity is changed
- Therefore, it is essential to return to the urine laboratory **within two hours** after sampling (or even faster)
- If, exceptionally, this cannot be fully met, **storage in a refrigerator** should be used (unlike for other specimens)

URICULT type devices

- The purpose of these units is **to totally eliminate the time lag** between the collection of urine and the beginning of cultivation. The urine is sampled and a special tool with cultivation media is placed in it. Then the urine is removed and the media start to be cultured (sometimes without sending to the lab, if a small incubator is available for it)
- On these plates, however, the microbes are **difficult to diagnose**. This method is therefore doesn't apply, as it was originally expected. In its use of large regional differences.
- If it is used, it is necessary to strictly follow **the correct procedure**

How to use an URICULT



- The cap with culture media should be **screwed out carefully** (to let the cap in the air during sampling)
- Urine midstream is used for **filling in the Uricult container to 3/4** (directly or from a sterile container).
- **The device with cultivation media is placed into the urine in the container**
- After several seconds **the device is removed**
- **Excess urine is left to drain** on the bottom of the plates, then sucked out with filter paper (do not touch the media)
- **The urine is removed from the vessel** including the remaining drops
- *Exceptionally it is possible to perform the sampling by placing the both sides of the media in the stream of urine*

Urine specimen processing

Qualitative and semiquantitative urine examination

- When a **quantitative examination** of the urine is diluted and given on a few of the culture media.
- At **semiquantitative examination,** urine is not diluted, but a calibrated single use loop on is used. The examination is less laborious, but also less accurate.
- Of course, **not only quantity is assessed**, but also the normal way to diagnose the microbe is used.

Semiquantitative processing I

- A **calibrated plastic loop** for 1 µl is used
- That means that when it is placed into the water or a liquid with similar surface tension **just one microlitre is kept in the „eye“ of the loop**
- This microlitre is inoculated to one **half of blood agar plate** (in practical session: on a **total plate**)
- After that it is **normally incubated** (24 h, 37 °C)
- The other day **colonies are counted**. According to the number of colonies the result is interpreted
- *In our laboratory we use now chromogenic medium instead of formerly used Endo or McConkey agar*

Bacteria on a chromogenic medium

Foto O. Z.

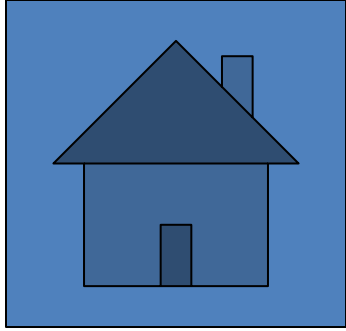
Semiquantitative processing I

- **Number of colonies** after incubation corresponds to the number of CFUs in 1 µl of original urine
  - CFU = **c**olony **f**orming **u**nit: one microbe, a pair, a short chain, a small group. In practice we neglect the difference between a microbe and a CFU, so we say that we count microbes when we really count CFUs
- If the number of colonies approximately corresponds the number of microbes in 1 µl of original urine, then the number of colonies × 1000 corresponds the number of microbes in 1 ml of original urine. 10 colonies – 10^4 microbes in one millilitre, 100 colonies – 10^5 microbes/ml

Automated culture systems

- Some companies now offer **automated culture systems**, which detect positivity already after four hours and they even refer the antibiotic sensitivity (Italian system UroQuick). Some, especially the private laboratory systems welcome it and base their microbiological examination of urine on this system.
- However, this approach is **very risky**, because the determination of the antibiotic susceptibility testing without specifying the type of bacteria is very treacherous. If there is such a system combined with the classic diagnosis, the damage is not necessary. However, **it is unacceptable to use such a system without its results being interpreted the microbiologist** (e.g. location of the instrument into biochemical laboratories).

Urine
Basic diagnostic schedule



- Day 0: start of culture only
- Day 1: result of primary culture of specimen on BA, EA/URI, expedition of all negative results, pathogen testing
- Day 2: expedition of positive results, if bacterial susceptibility is sufficient (if not, → more tests)
- Day 3: expedition of remaining results

Result interpretation, prevention and treatment of UTIs

Urine – result interpretation

- There is **no common flora**, nevertheless, in elderly often asymptomatic bacteriuria (ABU), it is not necessary to treat it
- Differentiation of **contamination, but also colonisation** (especially in cathethrized patients) is often very difficult, often possible only based on clinical situation (the microbiology finding itself is not sufficient)
- **Among pathogens**, the most common is *Escherichia coli*, more Enterobacteriaceae, yeasts, enterococci, *Streptococcus agalactiae*, *Staphylococcus saprophyticus* etc

Interpretation of urine examination I

- **When we find one microbe, it is valid that**
- **Quantity over 10^5** microbes in 1 ml is considered likely uroinfection. In elderly it nevertheless might be a colonization
- **Quantity 10^4–10^5** is borderline. It is not sure whether the specimen was taken properly (e. g. in babies) it is rather considered a contamination. It is rather important in men and children. Antibiotic susceptibility is nevertheless tested
- **Quantity < 10^4** is considered a contamination
- Not valid for punctured and cathethrized urine

Semiquantitative urine evaluation at finding one microbe

Number of colonies	Number of CFU (bacteria) in 1 µl of urine	Number of CFU (bacteria) in 1 ml of urine	Evaluation (valid for 1 bacterium)
Less than 10	Less than 10	Less than 10^4	Contamination
10–100	10–100	10^4–10^5	Borderline
More than 100	More than 100	More than 10^5	Infection

Interpretation of urine examination II

- **In case of finding two microbial species approx.:**
- **Quantity < 10^5** is evident contamination
- **Quantity > 10^5** is borderline (unsure)
- **In case of finding three microbial species:**
- Nearly always we take it as **contamination**
- **Exception:** one microbe in quantity > 10^5, two other microbes < 10^4 → first microbe is considered pathogen, the other two contamination
- In practice we also take into account **what species of microbes** do we have (staphylococci use to be taken less seriously)

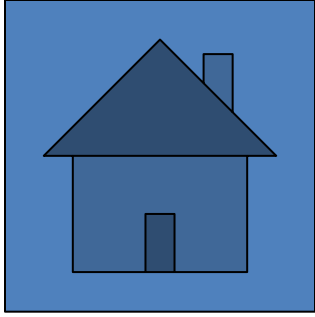
Asymptomatic bacteriuria (ABU)

- Only real actual infection that is causing the problem should be **treated** – not the mere presence of bacteria in urine (particularly in older people),
- However, there may be exceptions:
  - **pregnant women** – we treat even ABU because urinary infection can become a focus of a vaginal infection → infection during delivery
  - or **some other risk situations**, e. g. a person with immunodeficiency; here also that bacteria might be the source of infection of other organs

Treatment of UTI

- For **uncomplicated community** (= not hospital) cystitis sometimes plant extracts (cranberries) are sufficient.
- As to **antibiotics**, for cystitis suitable nitrofurantoin is suitable (does not concentrate in the blood, but in the urine). Another option is to co-trimoxazole, amoxicillin, second generation cephalosporins, doxycycline, and more
- For **hospital cystitis** treatment should be chosen according to the susceptibility (but this is useful even for outpatients)
- For **pyelonefritis** (inflammation of the renal pelvis) the antibiotics must penetrate not only into the urine, but also into the renal tissue. Nitrofurantoin or norfloxacin is therefore not applicable. Targeted treatment for the causative agent is used.

Prevention of UTIs



- **Very effective preventive techniques:**
  - To urinate after sexual intercourse (especially in women)
  - To prefer hormonal contraception to barrier methods
  - Change frequently menstrual devices
  - Do not use spermicide gels, creams, gels and perfumed napkins
- **Completely wrong and dangerous techniques:**
  - Excessive hygiene
  - Overuse of so called disinfection gels and soaps
  - Frequent bathing in bathing foams

According to the „Recommended guideline for antibiotic treatment of community infections of kidneys and urinary tract infections in the primary care“

Infection of reproductive organs – introduction

Importance of this group of infections

- Infections of sexual organs are also **quite frequent infections**
- The problem is that **it is difficult to assess how frequent they really are**. Ill people often try self-treatment and remain hidden to the healthcare, because they are ashamed and they have shame to speak about it (including with a doctor)
- Another problem is **difficult implementation of effective measures for treatment and prevention**. Also in diseases where sexual intercourse does not play the main role (e. g. vaginal mycoses) it is useful to treat both (all) partners

Normal situation of genital organs

- In normal situation there are no microbes:
  - **In females** in uterus, tubas, ovarias
  - **In males** in prostate, ducts, testes
- Specific normal flora is in **vagina** (lactobacilli, some more aerobic and anaerobic microbes). Also microflora of **distal part of urethra** is partially specific
- **Vulva** is the borderline between vaginal and skin flora
- In males also **prepuce bag** is specific is specific, besides skin flora there are also e. g. non-pathogenic mycobacteria etc.

Classification of sexual infections

- **The classic sexual diseases** are transmitted almost exclusively by sexual way. They are a subject of registration and reporting under the special laws. For us, this includes primarily **gonorrhoea** and **syphilis**
- **Other infections of genital organs** are those that affect the sexual organs, but sexual transmission is not the only or even the most important
- There also exist infections **transmitted sexually**, but not affecting directly sexual organs (hepatitis B, AIDS, etc.)

There exists the term "sexually transmitted infections" – STI (formerly STD – sexually transmitted diseases). The content of the term is rather changeable by its user.

Classical sexual diseases

Gonorrhoea	***Neisseria gonorrhoeae* („gonococcus“)**	**Common also in Europe**
Syphilis (lues)	***Treponema pallidum***	
Chancroid (ulcus molle)	*Haemophilus ducreyi*	**In Europe rare, from other countries**
Granuloma inguinale	*Klebsiella granulomatis* *(ex: Calymmatobacterium)*	
Lymfogranuloma venereum	*Chlamydia trachomatis* serotypes L_1, L_2, L_3	

Other agents of sexual infections – 1

- **Human papillomavirus** (related to cervical carcinoma – almost types 16 and 18, other types – causing condyllomata acuminata etc.)
- **Herpes simplex virus** type 2, eventually also type 1
- **Virus of molluscum contagiosum**
- ***Chlamydia trachomatis*** – serotypes D to K
- ***Ureaplasma urealyticum, Mycoplasma hominis*** and more urogenital mycoplasms
- ***Gardnerella vaginalis, Mobilluncus mulieris***, anaerobic bacteria (bacterial vaginosis – more later)

Other agents of sexual infections– 2

- **Enterobacteriaceae, streptococci, enterococci, staphylococci** and more agents of so called areobic vaginitis
- **Yeasts** especially of genus *Candida*
- ***Trichomonas vaginalis***
- **Pubic lice** also maybe classified here, although directly reproductive organs are not attacked

Interpretation of „positive“ findings

- Like the other places with normal microflora (intestine, the oral cavity) **vagina can also be considered an ecosystem**. Its stability is influenced both by microbes and the host-side factors
- In many cases **the culture positivity itself is no reason to treatment**, what's important is the clinical context. This concerns in particular the anaerobic bacteria, gardnerel, urogenital species of *Mycoplasma* and *Chlamydia*
- **Microscopy** is often useful for the interpretation. Unlike the culture we see **ratios** of bacteria

Bacterial vaginosis (BV)

- **Bacterial vaginosis** is a condition where the normal vaginal flora in the vagina is diseased and vagina contents larger amounts of *Gardnerella*, *Mobiluncus*, and anaerobic bacteria. All of them **may be in the vagina also normally, but usually not so many**
- **We can't determine a unique causative agent**
- **Almost no leucocytes are present.** Some bacteria blocate their migration to the inflammation site. On the other hand, In microscopy we see epithelial cells covered with bacteria – **clue cells**
- **Treatment:** metronidazole, probiotics

Nugent score

- Some laboratories use **microscopy of vaginal smear** for counting of so called Nugent score. Here „plus points“ are counted for gardnerella-shaped bacteria (tiny gram-labile rods) or mobillunci (small curved G– rods) and „minus points“ for lactobacilli-resembling bacteria. **Score over 10 is nearly sure for vaginosis**

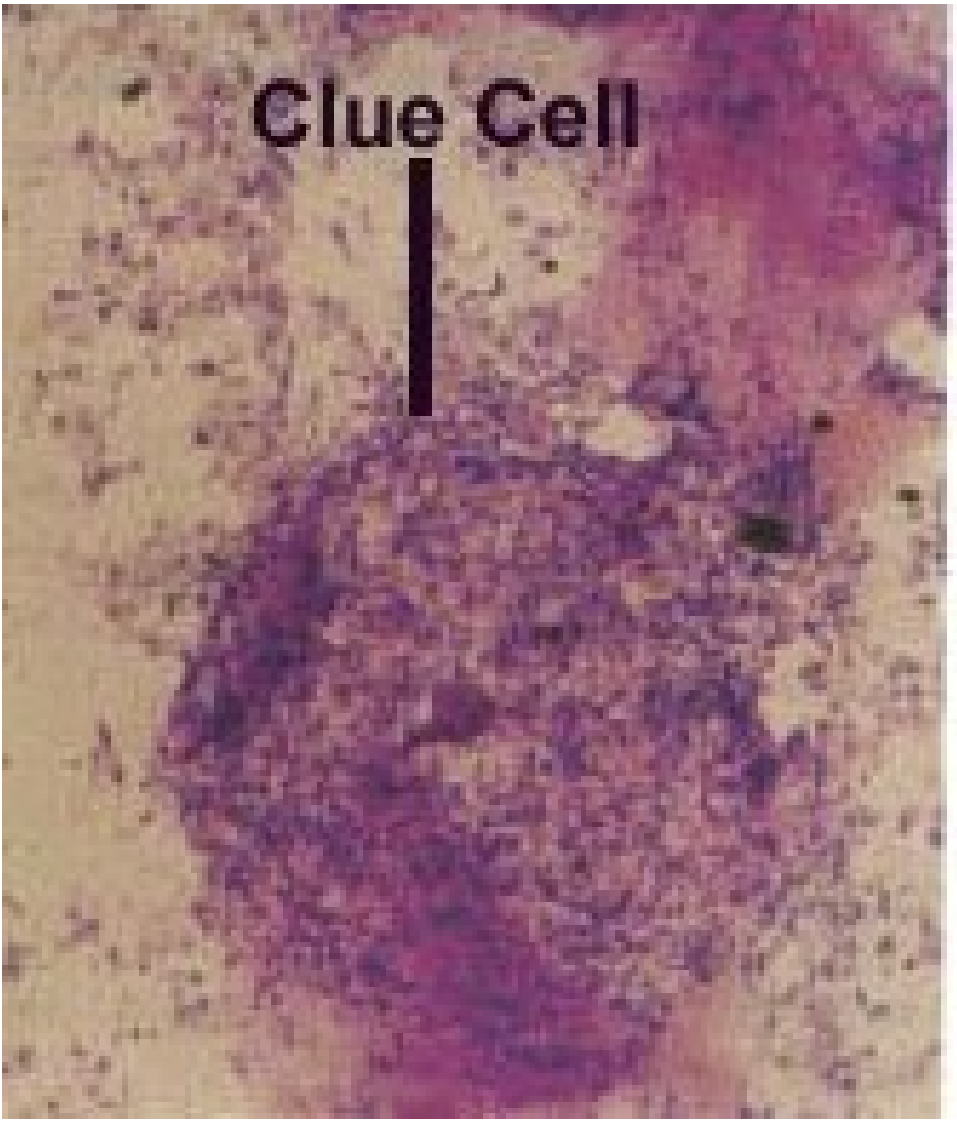
Nugent score more concretely

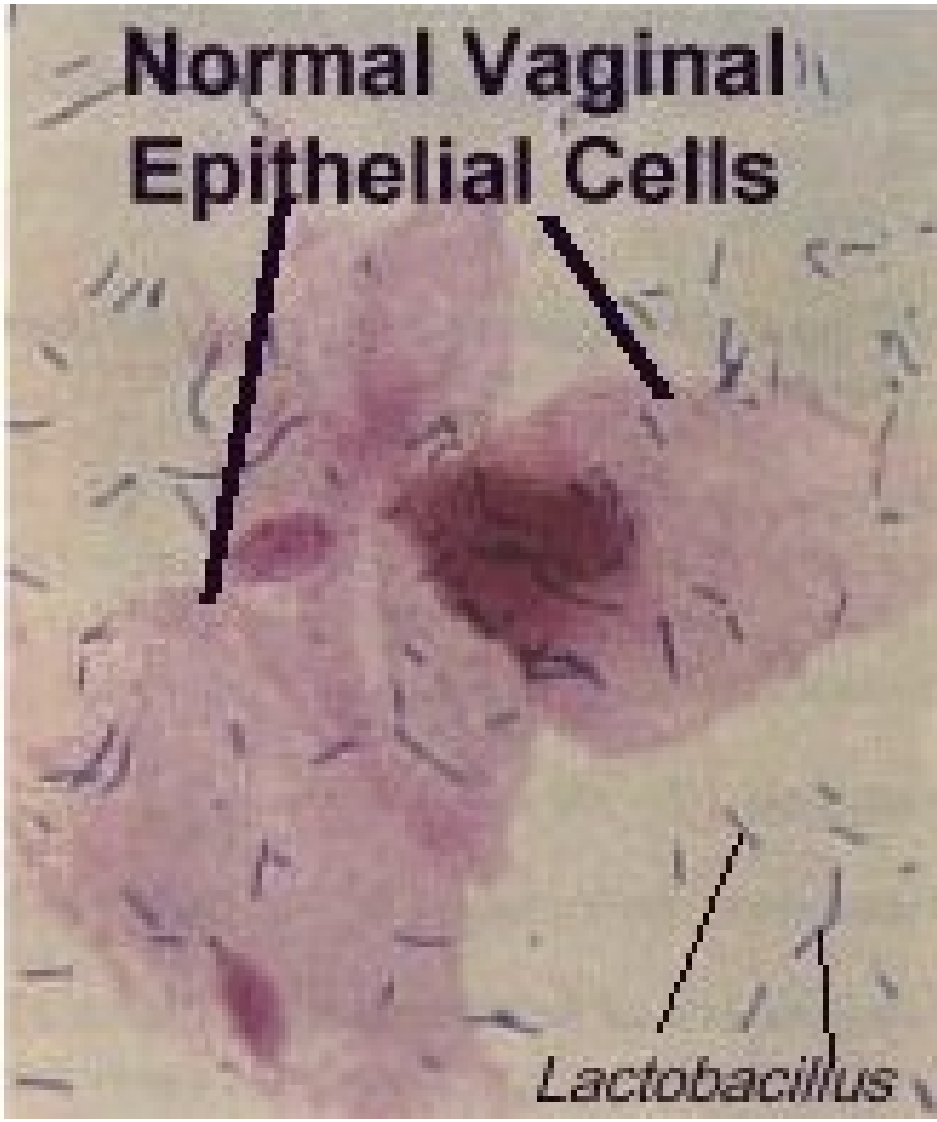
Due to the fact that it is a microscopic and not the culture proof, we work with so-called morphotypes.

For example, bacteria belonging to the "morphotype Lactobacillus" may no Lactobacillus, but it is very likely

- **Morphotype Gardnerella/Bacteroides:** not present = zero point, + = one point, ++ = two points, +++ = three points, ++++ = four points
- **Morphotype Lactobacillus:** the contrary: not present = four points, positivity ++++ = zero points
- **Curvet Gram-labile rods:** none = 0 points, + or ++ = one point, +++ or ++++ = two points

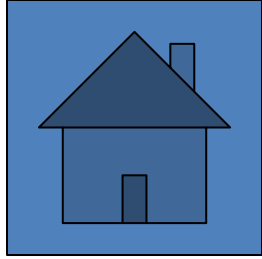
Clue cells





http://www.kcom.edu/faculty/chamberlain/Website/lectures/lecture/image/clue2.jpg

Aerobic vaginitis (AV)



- Besides bacterial **vaginosis** there also exists classical (i.e., containing leucocytes) bacterial vaginitis (**colpitis**; however, the concept of **vaginitis**, an incorrect combination of Latin and Greek, unfortunately took and used)
- However, it is **very difficult to distinguish the agent** of the inflammation from the accidental discovery or colonization of the vagina
- **Most commonly we find** *Enterobacteriaceae*, enterococci, *Streptococcus agalactiae, Staphylococcus aureus*
- **Treatment** depends on the presence of symptoms, with the exception of *Streptococcus agalactiae* (outside of pregnancy is recommended rather woman healing due to the transfer to the newborn; in pregnancy itself we do not treat, but the delivery is protected)

Sampling and examination in genital infections

Possible specimens in genital infections – anatomic classification

- **Vaginal swab** – usually from the rear side of vagina, using gynaecologic mirrors, must not be contaminated by the microbial flora of the vulva
- **Cervical swab** also using gynaecological mirrors
- **Urethral swabs** in both genders
- **Swab from penis, prepuce, glans** in men
- **Ejaculate** (or swab from ejaculate)
- **Swab from labia** in females
- **Invasive specimens** (content of cyst etc.)

Possible specimens in genital infections – according to the causative agents

- **Amies swab** – for aerobic bacteria, *Gardnerella,* anaerobic bacteria, event. also urogenital mycoplasms (some laboratories use special media for mycoplasms)
- **Dry swab** is almost used for non-cultivation detection of antigens and DNA, i. e. in chlamydias, papillomaviruses etc.; if we wish to have a specimen from deeper layers of the mucosa, we would use a brush
- **E-swab** may eventually replace both previous swabs (as the producer says, it enables both culture and PCR)
- **C. A. T. swab** is for yeasts and *Trichomonas*
- **Smears** may be sometimes very useful
- **Clotted blood** is used for antibody detections (e. g. syphilis)

Smears from vagina or urethra

- It is a situation where the clinician directly makes a smear of secretions on the slide. **Caution – If the slide is not sterile, the swab should not be used for culture**
- Classic variant – **microscopic appearance of vaginal microflora (MAVM)**
  - We send **two slides** with vaginal smears
  - One is Gram-stained for bacteria, epithelial cells, WBCs, yeasts etc.
  - The other is Giemsa stained (mainly because of *Trichomonas*)
  - We evaluate both **quantity of individual formations**, and also **final appearance** of the preparation
- In **gonorrhoea** we rather send urethral and cervical swabs, an usually only one slide to Gram stain. More in the material to *Neisseria*

Normal microflora: epithelia, laktobacilli (Döderlein bacillus)

Giemsa

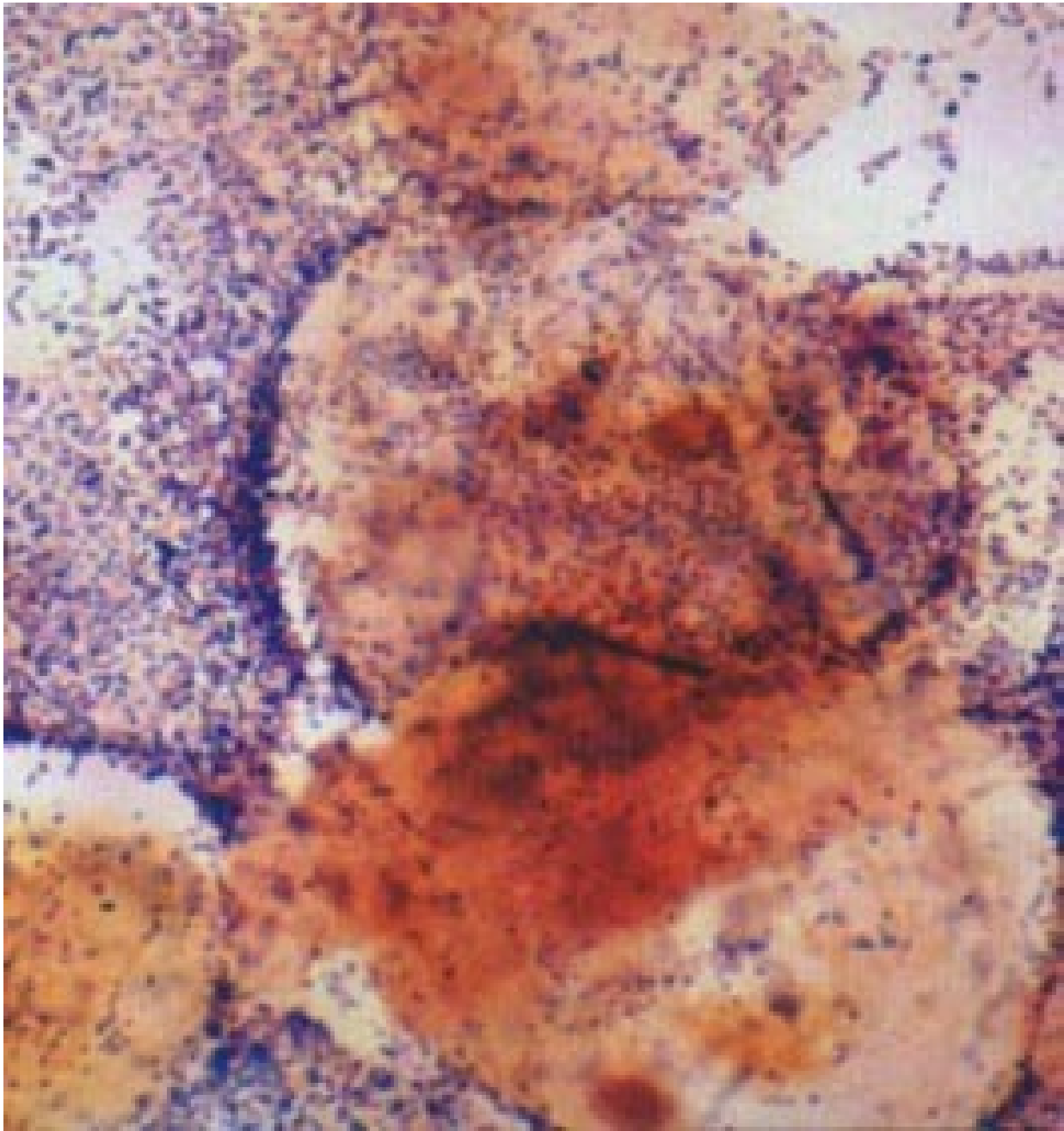
In reality we may also consider normal mixture of lactobacilli with other microbes, if clinical symptoms are absent.

http://en.microdigitalworld.ru

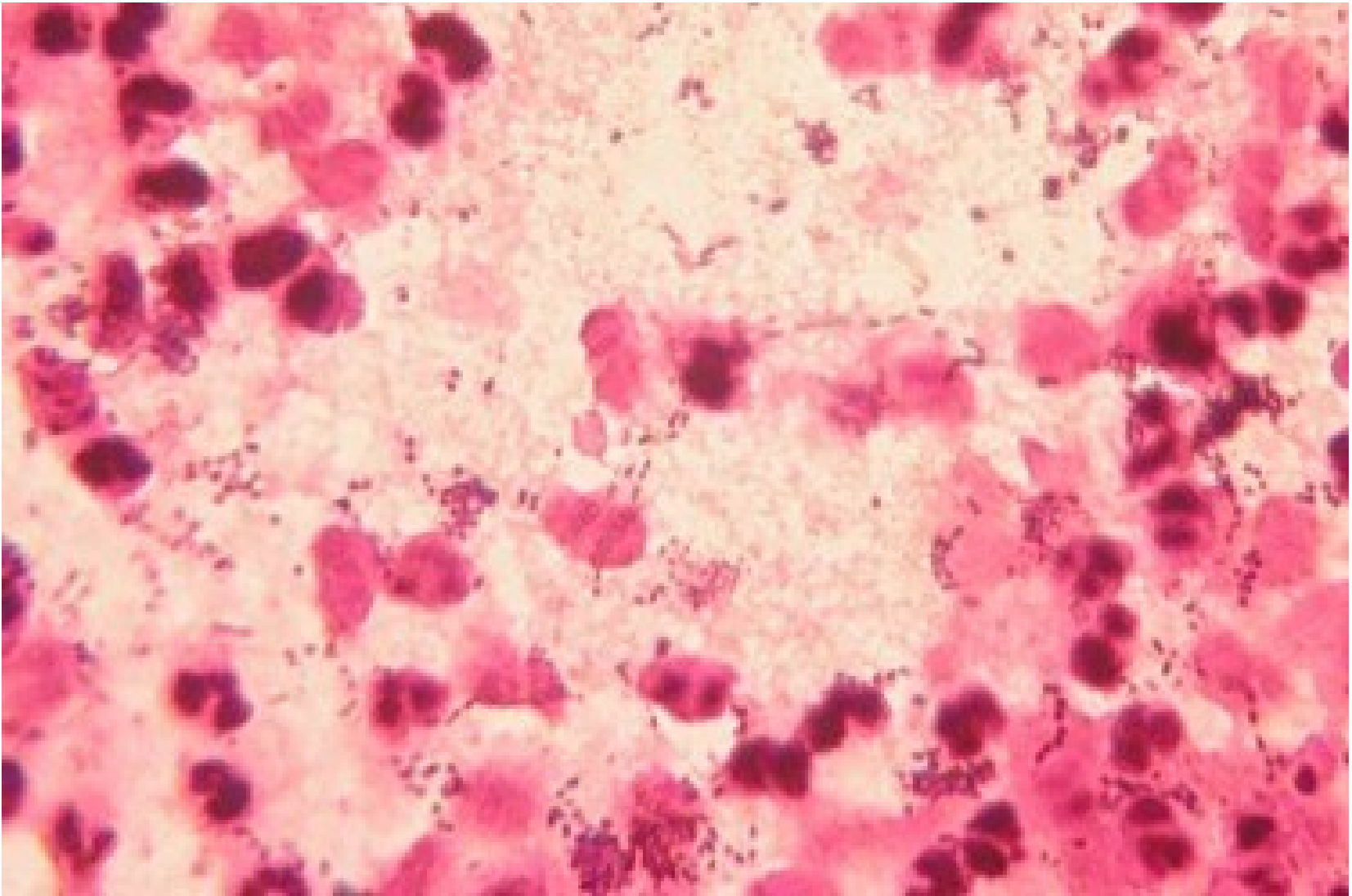
Picture of bacterial vaginosis

(lactobacilli replaced by Gardnerella and mobilunci and other bacteria, common clue cells – bacteria adhered on epithelia)

Gram



Aerobic vaginitis (unlike for vaginosis, white blood cells are present)



Gram

http://en.microdigitalworld.ru

Gonorrhoea

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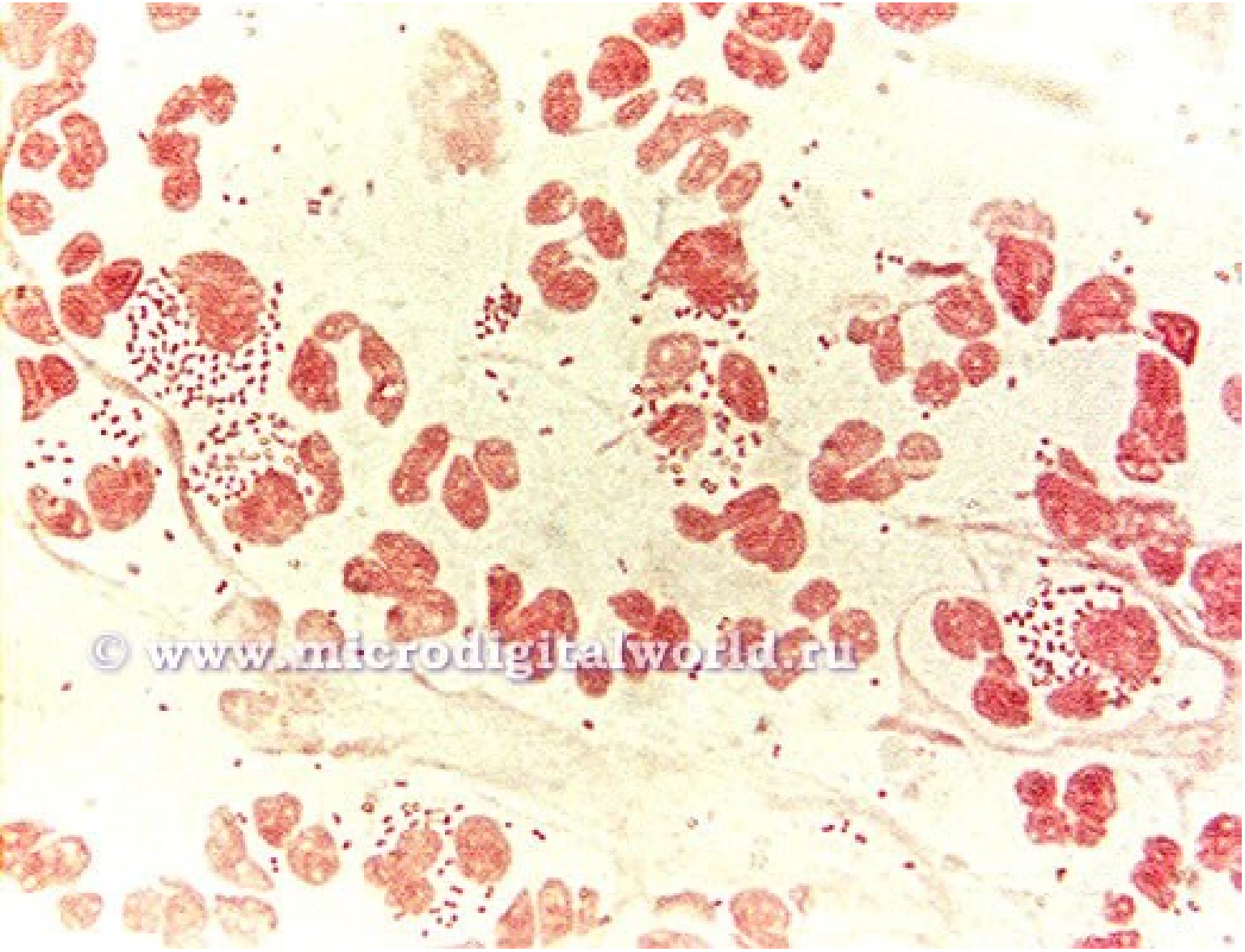

Gram

Trichomonosis

Giemsa

Vaginal mycosis

http://en.microdigitalworld.ru

Giemsa

End

http://manganime.animeblogger.net/wp-content/2006-04/HSGep2/_HSG%20ep%202%20Urine%20sample%2001.jpg